Exploitation of secondary metabolites by animals: A response to homeostatic challenges

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Synopsis We propose that the exploitation of the bioactive properties of secondary metabolites (SMs) by animals can provide a ''treatment'' against various challenges that perturb homeostasis in animals. The unified theoretical framework for the exploitation of SMs by animals is based on a synthesis of research from a wide range of fields and although it is focused on providing generalized predictions for herbivores that exploit SMs of plants, predictions can be applied to understand the exploitation of SMs by many animals. In this review, we argue that the probability of SM exploitation is determined by the relative difference between the cost of a homeostatic challenge and the toxicity of the SM and we provide various predictions that can be made when considering behavior under a homeostatic perspective. The notion that animals experience and respond to costly challenges by exploiting therapeutic SMs provides a relatively novel perspective to explain foraging behavior in herbivores, specifically, and behavior of animals in general. We provide evidence that animals can exploit the biological activity of SMs to mitigate the costs of infection by parasites, enhance reproduction, moderate thermoregulation, avoid predation, and increase alertness. We stress that a better understanding of animal behavior requires that ecologists look beyond their biases that SMs elicit punishment and consider a broader view of avoidance or selection of SMs relative to the homeostatic state. Finally, we explain how understanding exploitation of SMs by animals could be applied to advance practices of animal management and lead to discovery of new drugs.

Introduction

Physiology and foraging behavior of herbivores are partially governed by the presence of chemical defenses, or secondary metabolites (SMs) in plants. Many species of plants produce SMs that can inhibit the growth of microbes, fungi, and other plants and typically have negative physiological and behavioral consequences following ingestion by animals; hence they are often referred to as defenses. SMs ingested by animals can cause loss of weight and failure of organs, alter metabolic rates, reduce digestibility of nutrients, compromise the expenditure of energy and even result in death, depending on the type and amount of SM consumed (Cheeke and Palo 1995; Dearing et al. 2005; Sorensen et al. 2005c; Froberg et al. 2007).

As SMs can be acutely or chronically toxic to herbivores, many herbivores have developed strategies that minimize the effects of the SMs they consume. Complete avoidance is typically not possible due to the ubiquity and diversity of SMs. Therefore, herbivores, which we broadly define as animals consuming plant matter (e.g., fruits, stems, leaves, roots, bark), will use a combination of behavioral and physiological mechanisms that minimize concentration of the SMs in the blood and tissues. For example, herbivores may consume small amounts of a variety of plants so that toxic levels of any single SM can be avoided (Freeland and Janzen 1974; Provenza 1995, 1996; Dearing and Cork 1999; McLean and Duncan 2006). Herbivores may also ingest smaller, more frequent meals and

From the symposium ''PharmEcology: A Pharmacological Approach to Understanding Plant-Herbivore Interactions'' presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2009, at Boston, Massachusetts.
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Integrative and Comparative Biology, volume 49, number 3, pp. 314–328
doi:10.1093/icb/icp046
Advanced Access publication June 22, 2009

thereby minimize the dose of SMs in any single meal (Wiggins et al. 2003; Sorensen et al. 2005a). Finally herbivores may reduce exposure to SMs through biochemical mechanisms that limit the absorption and distribution and maximize the metabolism and elimination of ingested SMs (McLean and Duncan 2006; Sorensen and Dearing 2006; Sorensen et al. 2006).

Given the potentially harmful consequences of many SMs to herbivores, it is logical to hypothesize that herbivores are deterred, not rewarded, from ingestion of SMs (Sullivan et al. 2008). Nevertheless, some animals intentionally consume SMs and benefit from it. For example, humans have a long history of exploiting the biological activity of SMs for medicinal purposes (Johns 1990; Cotton 1996). Although indigenous peoples have a much longer history of using medicinal plants, the first written accounts of SMs for medicine are contained in the Ebers papyrus, circa 1500 B.C. (Klaassen 2001). Plant-based medicines continued to be used throughout history, but it was only in the 19th century that active SMs were isolated and characterized. Some of the most influential discoveries of SMs with therapeutic uses include the isolation of morphine from poppies in 1820; quinine from *Cinchona* bark in 1841; ephedrine from *Ephedra* species in 1897 and tubocurarine from *Chondrodendron tomentosum* in 1935 (Sneader 1996). SMs or their derivatives account for nearly half of small-molecule New Chemical Entities approved on the market since 1994 (Newman and Cragg 2007), with others currently in clinical trials (Butler 2008; Harvey 2008). Although the medicinal use of SMs is well documented, humans are not unique in the animal kingdom for exploiting SMs for benefit (Huffman 2003, 2007a). Janzen (1978) was the first field biologist to suggest animals may benefit from SMs as anti-parasitic agents. In his seminal paper, he provided numerous anecdotal examples of self-medication with plants from a diverse array of wild animals, including herbivores, omnivores, and carnivores. More striking are the scientific descriptions of nonhuman primates ingesting plants with known pharmacologically active SMs, possibly as treatment of parasitic disease. The question that arises, is how a SM that presumably evolved, at least in part, as a deterrent, would be chosen by animals as a reward (Huffman and Seifu 1989; Sullivan et al. 2008)? Although ecologists have described how insects with high resistance to SMs can exploit SMs as cues for finding food, mates, or sites for oviposition (Feeny 1992), there are few explanations addressing why animals that have not co-evolved with SMs would exploit SMs that are potentially toxic.

We propose that the exploitation of SMs can be explained through understanding the homeostatic challenges animals face. All organisms are in a constant battle to stay in a state of equilibrium, or homeostasis, with their environment. For herbivores, the homeostatic state can be perturbed by a diet that can offer nutritional deficits and potentially toxic SMs (Provenza and Villalba 2006). Several studies demonstrate that homeostasis in herbivores can be altered by ingestion of SMs. For example, herbivores may have limited tolerance for SMs such that they cannot consume enough plant matter to maintain energy balance (Sorensen et al. 2005b,c). In addition, the homeostatic state of herbivores may be perturbed by internal or external factors, other than SMs, that put pressure on the state of health. SMs may provide the "treatment" for factors that challenge the homeostatic state. The homeostatic perspective suggests that selection of diet may be guided not only by avoidance of SMs, but, in some cases, by selection for SMs that ameliorate other challenges. In this review, we describe the general conditions in which potentially toxic SMs might be actively chosen by herbivores and other animals whereby homeostasis is achieved. We provide various predictions that can be tested when considering foraging behavior under a homeostatic perspective. Finally, we explain how understanding exploitation of SMs by animals could advance animal-management practices and lead to discovery of new drugs.

Homeostatic perspective: establishing the probability of exploitation of SMs

Imagine an herbivore in a tropical habitat. This animal, like most, is infected with parasites that impact immunity and deplete energy. It is also the mating season and resources are needed for ensuring reproductive success. In addition, the scent of a predator is in the air, which elevates stress hormones. These challenges can negatively affect the internal balance of energy and nutrients and thus compromise the health of the animal. The intake of proper energy and nutrients can help animals return to well-being, or homeostasis. Unfortunately, achieving internal homeostasis through feeding is not a simple task. Herbivores must meet demands for energy and nutrients by choosing the "right" diet in an environment containing 25–50 plant species. These plants differ in their concentrations of energy, protein, minerals, vitamins, indigestible matter (e.g., fiber, cellulose) and SMs. Plants contain nutrients that

can lessen the costs of a challenge. In addition, the SMs that are potentially toxic may, in certain doses, alleviate the costs of many external challenges. For example, many SMs and their derivatives are used by humans to combat bacterial and parasitic infections, cancer, and a variety of other ailments (Harvey 2008). Likewise, wild animals may exploit the bioactive properties of SMs that provide "treatment" or "self-medication" for their own ailments (Huffman 1997, 2003).

The notion that animals experience and respond to different physiological states by selecting plants containing medicinal properties provides a relatively novel perspective to explain foraging behavior in animals. The ecological literature devoted to plant–herbivore interactions is full of examples demonstrating how selection of diet is driven by avoidance of SMs (Freeland and Janzen 1974; Howe and Westley 1988; Lindroth 1988; Freeland 1991; McArthur et al. 1991; Rosenthal and Berenbaum 1992; Cheeke and Palo 1995; Dearing and Cork 1999; Foley et al. 1999; Karban and Agrawal 2002; Dearing et al. 2005; McLean and Duncan 2006). We aim to establish a theoretical framework, based on various examples in the literature, suggesting that, in some cases, animals exploit the bioactive properties SMs and thereby mitigate challenges like infection, reproduction and thermoregulation. We stress that knowledge of the relative cost of each challenge and toxicity of each SM is important in determining the internal and external conditions that warrant exploitation of SMs.

We predict that the likelihood of SMs being exploited by animals as a natural treatment against a challenge is dependent on both the cost of the challenge and the therapeutic index of the SM in a particular animal. The therapeutic index is defined as the ratio between the concentration of a given chemical resulting in toxicity and the concentration providing therapy (American Heritage Medical Dictionary, Fig. 1), and provides a general measure of the toxicological cost for a chemical. The therapeutic index of SMs consumed by herbivores is influenced by the chemical properties of the SM (e.g., solubility, size) and the capacity of the herbivore to detoxify and eliminate the SM. In general, if a SM is readily absorbed and has a slow rate of detoxification and elimination, then very small amounts of intake could result in concentrations in the body that are toxic and the SM is likely to have a narrow therapeutic index (Fig. 1). If, however, the SM is not readily absorbed and the animal has enzymes that rapidly detoxify and eliminate the SM, then the SM is likely to have a wide therapeutic index and may be considered less toxic (Fig. 1).

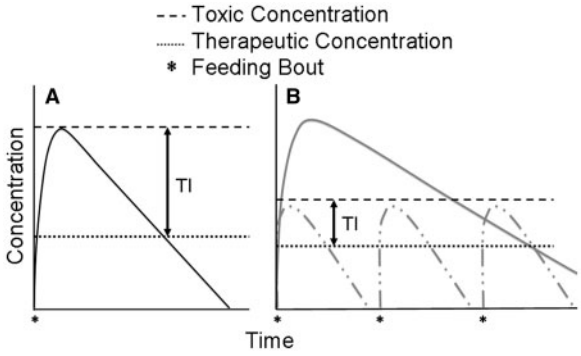

Fig. 1 The therapeutic index (TI) of an ingested SM (or drug) is dependent on the ratio between the therapeutic concentration (dotted line) and the toxic concentration (dashed line) in a particular animal. The concentration of any ingested SM (or drug) following a feeding bout (∗) increases as it gets absorbed, then decreases as it gets metabolized and eliminated. (**A**) A theoretical animal consuming an ideal dose of a SM in which concentrations remain above the therapeutic concentration, but below the toxic concentration, thus within the therapeutic window for the longest period of time. (**B**) Two theoretical animals that differ in their exposure to the same ingested dose of a SM with a narrow TI. The solid grey curve represents an animal that has high absorption and slow detoxification of the SM from a single feeding bout resulting in concentrations that surpass toxicity (dashed line). This animal should avoid the SM or consume very small amounts even if it is therapeutic at some concentration. The dash dotted grey curve represents an animal with lower absorption and faster detoxification of this same SM resulting in concentrations below toxicity. This animal may have several feeding bouts (∗) to achieve concentrations of the SM above the therapeutic concentration for longer periods of time.

Drawing from the principles of the therapeutic index, we can predict the probability that an animal will exploit a SM for treatment of a particular challenge. That probability is determined by the relationship between the cost of a challenge and the toxicity of the SM (Fig. 2). The cost of a challenge is determined by the impact a challenge has on the fitness of an animal, whereas toxicity is determined by the therapeutic index of the SM in a particular animal. For example, the cost of parasites is expected to increase with increasing parasite load and a SM is expected to have a wider therapeutic index in the diet of a specialist herbivore than of a generalist or an animal naïve to that diet. The ultimate "goal" for the animal is to regulate homeostasis such that a balance is kept between minimizing the cost of the challenge and minimizing toxicity. We predict that SMs with a wide therapeutic index could be exploited by animals incurring less costly challenges, whereas SMs with a narrow therapeutic

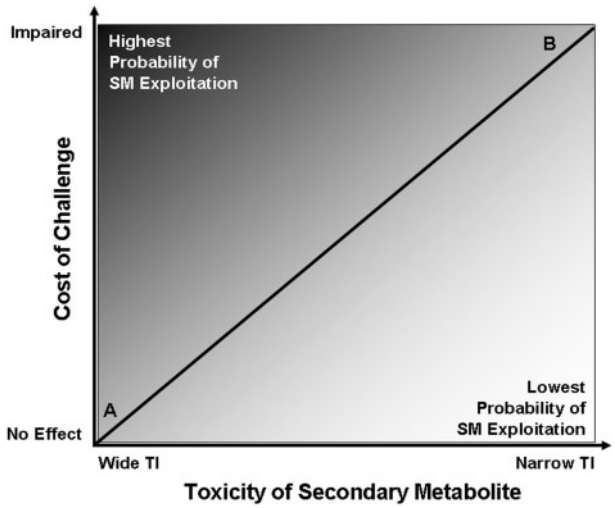

Fig. 2 The probability that an animal will exploit a SM is a function of the relative cost of a challenge (e.g., parasites, reproduction, thermoregulation) and the toxicity of a potentially therapeutic SM. The wider the relative cost between the challenge and the toxicity of an SM, the greater the probability the SM will be exploited by an animal for self-medication against the challenge. SMs with wide therapeutic indices (TI) are considered to have lower toxicity than SMs with narrow TIs. Darker shading represents higher probability of exploitation of SMs. (**A**) A representative case in humans in whom lack of alertness has a relatively low cost and the intake of caffeine represents treatment with a relatively nontoxic SM due to a wide TI. (**B**) A representative case in humans in whom cancer represents a high-cost challenge and paclitaxel represents treatment with a very toxic SM due to a narrow TI.

index would be exploited by animals incurring more costly challenges. Costs of challenges and toxicity are dynamic and difficult to measure. Therefore, selection of diets by animals under varying challenges can be used as an indicator of the relative costs of challenges and the toxicity of a SM (Fig. 2).

Evidence for SM exploitation related to costs

Modern medicine provides the most obvious evidence that exploitation of SMs by animals is related to the cost of a challenge versus the therapeutic index of a SM. It may be supposed that a wide therapeutic index is always essential for drug therapy in humans, but this is not necessarily the case. Cytotoxic drugs, like paclitaxel (a SM), are used to treat life-threatening (i.e., high cost) cancers despite their severe toxicity associated with a narrow therapeutic index. In contrast, reduced alertness (i.e., low cost) is widely treated with caffeine (a SM) because of its inherently low toxicity associated with a wide therapeutic index. We speculate that herbivores may act similarly: more toxic SMs may be consumed in desperate situations, whereas less toxic SMs may be consumed in the alleviation of noncritical challenges.

The following sections provide evidence that animals exploit the biological activity of SMs to mitigate the costs of parasitic infection and of reproduction and thermoregulation. We focus on these challenges because they are costly and there is at least one study demonstrating that animals can alleviate these challenges by exploiting the biological properties of SMs. Animals may also exploit SMs in reducing predation and mediating alertness. We focus on exploitation of SMs by homeotherms (e.g., mammals and birds) because they allow the best inference with uses of SMs for similar challenges in humans. Although herbivores are the most likely animals to exploit SMs due to their natural association with plants, we provide evidence that SMs in plants and animals can be exploited both by herbivores and nonherbivores.

Parasites

Ectoparasites

Ectoparasites represent the challenge most likely to be treated by SMs because ectoparasites are costly and animals do not actually ingest SMs to combat ectoparasites and therefore minimize toxicity (Fig. 2). Numerous studies have reported a cost of ectoparasites on host reproductive success and survival (Combes 2001). In response, several animals may reduce ectoparasite loads by exploiting SMs from plants or insects. For example, mammals and birds use the bioactive properties of leaves to line nests and fumigate ectoparasites (Lafuma et al. 2001; Hemmes et al. 2002; Rajasekar et al. 2006). Some animals apply the SMs from plants and insects onto fur or feathers as a defense against ectoparasites (Clayton and Vernon 1993; Douglas et al. 2001). Other species obtain the bioactive SMs from plants and animals in their saliva by chewing on chemically defended plants and animals and then anointing fur or feathers with their saliva (Weldon et al. 2003; Huffman 2007b). We predict that the wide use of SMs against ectoparasites by a variety of animals is due to the low toxicity of SMs, afforded through low levels of exposure to SMs by the host. The placement of leaves in the nest or anointment of SMs to fur and feathers does not involve ingestion, and thus the chemical is not absorbed. It is possible that hosts are exposed to SMs through inhalation of volatile SMs in nests or absorption across the skin or in the mouth during chewing. However, some plants

do not contain volatile compounds and thus would not be inhaled and exposure following topical or oral absorption is expected to be much lower than absorption across the gut. In cases in which ectoparasites are not costly (Munger and Karasov 1991; Gallizzi et al. 2008; Heylen and Matthysen 2008), we predict animals will not exploit SMs as there is little benefit derived from the costs associated with even low levels of SM exposure or costs incurred by the process of obtaining nesting or anointment material. However, as costs of ectoparasites increase along a continuum, we predict that choice of certain bioactive SMs will also change.

Endoparasites

Endoparasites represent another costly challenge, as intestinal parasitic infection is the rule rather than the exception in animals. Intestinal parasites, specifically nematodes, negatively affect body condition and fecundity in animals (Irvine et al. 2006; Reed et al. 2008). Intestinal parasites can be treated by SMs such as condensed tannins that are ingested, but not absorbed, and therefore may have low toxicity in the host (i.e., wide therapeutic index). The potentially high cost of infection and potentially low toxicity of SMs that are not absorbed should result in a large number of animals exploiting SMs in treatment of intestinal parasites. Indeed, several studies demonstrate that SMs with low absorption are exploited. For example, parasitized lambs ingest enough tannin, a type of SM that is not absorbed, to reduce helminthoses (Lisonbee 2008; Lisonbee et al. in review). In addition, sheep with parasite burdens also manifest greater preferences for a tannin-containing food, but not when infection is terminated by dosing with ivermectin, an anti-parasitic drug (Villalba et al. in review).

We predict that absorbed SMs will have the highest toxicity and should only be used when costs of infection are highest and even then, they should be consumed in small amounts. Although there is very little empirical support for this prediction, the combination of several separate studies suggest that the exploitation of highly toxic SMs is rare and only occurs in wild animals with severe parasite challenges. For example, potentially therapeutic plants are eaten only occasionally by chimpanzees and typically in small amounts, but they increase in frequency in the diet during months when reinfection by parasites responsible for reduced health occurs (Huffman and Seifu 1989; Huffman 1997, 2003; Huffman et al. 1998). Moreover, some of the plants exploited by primates only during times of infection also treat blood-borne parasites, like schistosomiasis, in humans, suggesting that these plants contain SMs that are absorbed into the blood (Ohigashi et al. 1994; Huffman et al. 1998). Similarly, plant parts that are rarely eaten by the Kanyawara group of chimpanzees in the Kibale forest, Uganda (Krief et al. 2006) contain SMs with significant anti-malarial activities (Krief et al. 2004). The selective use of this plant, coupled with efficacy against a blood parasite, suggests that this plant contains SMs that are absorbed and potentially toxic and would only be exploited by animals under extreme parasitic challenges. These studies, and many others (Huffman et al. 1993, 1996, 1998; Huffman and Chapman 2009; Engel 2002), indicate an association between exploitation of SMs and infection and demonstrate the need for controlled experimental studies.

Several experiments on domestic animals offer guidance for the types of studies needed to empirically test exploitation of SMs for the treatment of parasites. To date, studies on feeding have focused on a single potential treatment for infection, such as tannins. However, researchers should use combinations of SMs found both within a single plant and among plant species, not single SMs, as this is natural choice for animals in the wild (Provenza et al. 2003). Moreover, a combination of SMs from mixed diets may better protect animals against a range of parasites. Because parasites can evolve resistance to any one anthelmintic, they are also likely to do so with a single SM (Waller 2006). Tannins, alkaloids, and terpenes each have anthelmintic effects by different mechanisms (Hocquemiller et al. 1991; Kayser et al. 2003), and may be more therapeutic against parasites in combination than alone. We expect that the best approach to treat infectious organisms with potential resistance to single SMs is for animals to consume a mix of plants with different SMs as a variety of SMs may deliver active ingredients with multiple actions on a broader array of parasites (Villalba and Provenza 2007). We urge ecologists to test SM exploitation in wild animals by carrying out future feeding experiments in environments that contain a variety of plants, each containing SMs with a different therapeutic index (Villalba and Provenza 2007).

Reproduction

We hypothesize that animals are capable of exploiting SMs that maximize or otherwise alter reproductive output (Huffman 1997). Costs of reproduction can be great and are related to hormonal regulation, metabolism, energy reallocation, impaired immunity,

and production of defenses against stress and toxicity (Harshman and Zera 2007). Given the importance of reproduction for the survival of individual animals, reproductive output needs to be maximized. The majority of studies investigating SM intake related to reproductive output indicate that SMs disrupt reproduction. For example, phytoestrogens in many plants bind to animal estrogen receptors and inhibit follicular development in females and decrease fertility in males (Dixon 2004). Several plants also contain SMs that are fetotoxic, resulting in abortions and birth defects in offspring (Gardner et al. 1998; Panter et al. 2002). These studies suggest that SMs do not benefit animals.

However, there is evidence that SMs may be used as cues in avoiding erroneous investment in reproduction under limited availability of resources. For example, SMs isolated from salt grass, naturally consumed by montane voles, decrease uterine weight, follicular development, and cause a cessation in breeding in voles (Berger et al. 1977). SMs in salt grass, and other grasses that suppress reproduction by voles, are only produced at the end of the vegetative growing season and thus provide cues for an imminent reduction in resources. Studies also suggest that animals may use SMs as cues to initiate reproduction when resources are most abundant (Leopold et al. 1976). For example, 6-methoxybenzoxazolinone (MBOA) in grass stimulates reproduction in montane voles and its presence coincides with the time of greatest availability of food for rearing of pups (Berger et al. 1981; Sanders et al. 1981).

Several studies also indicate that the intake of SMs may actually benefit reproductive output. For instance, tannins make protein unavailable for digestion and absorption until it reaches the more acidic abomasums of ruminants, thus enhancing nutrition by providing high-quality protein to the small intestines (Min et al. 2003). This bypass also enhances immune responses and increases resistance to gastrointestinal nematodes in ruminants (Min et al. 2003, 2004). This immune effect, along with a resulting increase in essential and branched-chain amino acids, improves reproductive efficiency in sheep (Min et al. 2001). Tannins may also improve fitness of offspring. For example, intake of tannins by reproducing lemurs resulted in increased milk production, which may benefit offspring (Carrai et al. 2003). In quail, isoflavones (SMs) in soy improved egg quality (Sahin et al. 2007) and the intake of SMs in rooibos tea increased body weight and egg production of quail (Jurani et al. 2008).

Thermoregulation

Several lines of evidence suggest that animals can also exploit SMs to maintain homeostatic body temperatures. First, hypothermia (low body temperature) and hyperthermia (high body temperature) can be potentially lethal in homeotherms (Gentilello 1995; White et al. 2007), like mammals, and therefore represents a costly challenge. Second, many of the physiological mechanisms used to maintain body temperature within the thermoneutral zone can be induced through SMs. For example, ingestion of capsaicin and caffeine (both SMs) reduces body temperature in a number of mammals (Ilback et al. 2007; Gavva 2008). The alkaloid from Wu-Chu-Yu, a Chinese herb, can minimize heat stress in warmer conditions by increasing heat loss and suppressing heat production through vasodilatation (Tsai et al. 1995). Other SMs such as the alkaloids in tall fescue and terpenes in pine trees can cause vasoconstriction and prevent the loss of heat (Oliver et al. 1993; Gardner et al. 1998), thus providing a thermal benefit in the cold. Finally, a recent study showed that at least one species of mammal can minimize the dissipation of heat through the intake of a natural diet containing SMs (McLister et al. 2004; Dearing et al. 2008). Intake of juniper elevated body temperature in woodrats. Furthermore, juniper can reduce thermoregulatory costs for woodrats acclimated to cold temperatures, whereas woodrats acclimated to warm temperatures experience increased thermoregulatory costs when consuming juniper (McLister et al. 2004). In addition, woodrats voluntarily consumed more juniper in the cold than in the warm (Dearing et al. 2008). We speculate that woodrats may exploit the SMs in juniper for warmth without increased energetic costs. Furthermore, we speculate that animals do not exploit plants to override the highly conserved adaptation of thermoregulation, but rather to supplement thermoregulation, such that SMs decrease the lower critical temperature that elicits a thermoregulatory response. This may be achieved through reduced heat loss via vasoconstriction or through heat production via the process of detoxification. We urge researchers to consider both availability and thermal benefits or consequences of SMs as factors that dictate choice of diet as temperatures increase and decrease throughout the season and in response to predicted increases in temperature associated with climatic change.

Predation

Predation is extremely costly to animals as the endpoint of this challenge is death. Therefore, we expect

less mobile animals that cannot readily flee from predators will exploit SMs with potentially narrow therapeutic indices (i.e., highly toxic) in reducing predation. The most well-documented example of SM exploitation as a defense against predators is the ingestion and sequestration of SMs by herbivores. Sequestration involves the bioaccumulation of ingested SMs into the herbivore's own tissues. Sequestration of SMs as a defense against predators is common in sedentary terrestrial animals (Duffey 1980; Camara 1997; Silva and Trigo 2002; Pasteels and Hartmann 2004; Vlieger et al. 2004; Daly et al. 2007) and occurs in marine mesograzers (Paul and Vanalstyne 1988; Pennings and Paul 1993), but is rare in more mobile animals. To our knowledge, there is only a single example of ingested SMs being used by birds and mammals as defense against predators. *Pitohuis*, a passerine bird, is thought to sequester the alkaloid, homobatrachotoxin, from beetles in their diet into the feathers and skin (Dumbacher et al. 1992, 2004; Dumbacher 1999). Exploitation of batrachotoxin has resulted in the avoidance of this bird as food by natives of New Guinea and likely deters other predators as well. We predict that the paucity of examples of SM sequestration in birds and mammals demonstrates that other predator-avoidance strategies such as cryptic behavior and physical "fight or flight" mechanisms are less costly than ingesting and sequestering SMs in these mobile animals. Moreover, body size, life span, metabolic needs and detoxification capacity of avian and mammalian herbivores may increase the costs associated with ingesting and sequestering high concentrations of SMs in tissues compared to the costs to insects.

Alertness

The stimulatory or depressant activities of SMs may mitigate a variety of less costly challenges, such as lack of alertness. Given the high cost of predation (i.e., death), animals are expected to benefit from maintaining a level of alertness (i.e., vigilance) through increased ability to detect predators. In addition, enhanced alertness may improve memory of quality habitats and foods. Animals may enhance alertness by consuming SMs that are stimulants. Although scientific documentation of animals affected by the stimulatory activities of plants is rare, we provide examples from humans and anecdotal examples from wild animals as evidence that SMs may enhance alertness.

Humans exploit SMs such as caffeine to increase alertness and reduce reaction times (Michael et al. 2008) and these same SMs can improve performance in nonhuman primates. For example, caffeine allowed sleep-deprived marmoset monkeys to maintain psychomotor performance (van Vliet et al. 2008) and nicotine increased attention and accuracy of tasks in rhesus monkeys (Bain et al. 2003). Although these studies do not investigate how SMs might influence cognitive ability of animals in the wild, there is anecdotal evidence that birds and mammals use SMs as stimulants much as humans do. One example from Africa stands out as perhaps the best documented use by animals of SMs as a stimulant. The plant, *Tabernanthe iboga* (Apocynaceae), is used in religious rituals in Cameroon (Pope 1969). Indigenous forest-dwelling peoples reportedly discovered the hallucinogenic properties of this plant by watching gorillas, wild boars and porcupines digging up and eating the roots, afterwards going into a wild frenzy (i.e., stimulated). The active principle in iboga is ibogaine, found in highest concentrations in the root. Ibogaine affects the CNS and cardiovascular system, along with tabernanthine and iboluteine, other active constituents in the plant. The stimulating effects are similar to caffeine and can increase stamina (Dubois 1955; Szumlinski et al. 2001). Although the initial effects of *T. iboga* may appear detrimental (e.g., frenzy), as concentrations decrease below hyperactive levels, animals may experience heightened alertness, similar to the effects of caffeine. Although stimulants can improve alertness in animals (Bain et al. 2003; van Vliet et al. 2008), and animals appear to select plants containing stimulants under some conditions (Huffman, personal comm.), studies are needed to link the exploitation of stimulatory SMs with improved attention or performance in natural situations.

Homeostasis and self-medication in humans

In the evolutionary perspective of SM–human interactions, SMs may have been selectively used to treat parasites, regulate neurotransmitter imbalances, or to address periodic dietary deficiencies in nutritionally constrained populations (Sullivan and Hagen 2002). Humans, like wild animals, cannot escape the selection pressures from parasites. The global burden of human disease resulting from helminth infections is comparable to that of tuberculosis or malaria (Mascie-Taylor and Karim 2003). Sullivan et al. (2008) pointed out that two of the most widely-used SMs today, nicotine (tobacco) and arecoline (betel nut), have well-demonstrated anthelmentic

properties in animals. They propose that the recreational use of nicotine and arecoline could be an evolved response to chronic parasitic infections in ancestral human populations. The traditional exploitation of SMs by humans may also have provided some relief from the stresses associated with harsh environmental conditions. Sullivan and Hagen (2002) noted that most of the commonly-used SMs today were originally exploited and domesticated by indigenous groups living in marginal environments—for example khat in the deserts of northeastern Africa, tobacco and coca in arid South American desert and alpine regions, and betel nut in southeastern Asian tropical rain forests. They also noted that, from the ethno-historical perspective, all of these SMs are stimulants used to manage hunger, fatigue, and thermal stress, which are inherent challenges to survival in inhospitable environments.

In humans, self-medication has been most closely studied in clinical populations with high rates of substance use such as depression and schizophrenia (Sullivan and Hagen 2002). Khantzian (1997) has proposed that self-medication in clinical population functions to regulate affect states. In an alternative model, Schneier and Siris (1987) have argued that self-medication is a homeostatic self-regulation of neurotransmitter imbalances. Specific examples of self-medication using an unprocessed SM include use of the indigenous betel-nut to ameliorate the symptoms of schizophrenia in a clinical population in Palau, Micronesia (Sullivan et al. 2007a, b). In an apparent convergence of indigenous self-medication and commercial drug development, xanomeline, a synthetic analog of the betel-nut alkaloid arecoline is currently in clinical trials as a novel agent for the treatment of schizophrenia (Shekhar et al. 2008).

Testing for exploitation of SMs

We urge ecologists studying plant–herbivore interactions, and animal behavior in general, to evaluate whether animals may be exploiting SMs in their environment to alleviate homeostatic perturbations caused by a variety of external challenges. We provide a sample of cases linking selective intake of SMs by animals with coinciding challenges. Additional examples will only be obtained if ecologists look beyond their biases that SMs elicit punishment and instead consider avoidance or choice of SMs relative to the homeostatic state. Ecologists should consider the balance between the cost of the challenge and the therapeutic index, or relative toxicity, of SMs as they relate to foraging behavior. The slow progress and acceptance of SM exploitation by ecologists certainly stems from a lack of experimental evidence. We recognize that the majority of examples suggesting SM exploitation are correlative and need experimental validation. We therefore offer a guide for ecologists on how to best identify and validate exploitation of SMs.

Ecologists should first focus on observations of animal behavior under conditions that are most likely to drive the evolution of exploitation of SMs. For example, high levels of infection and high reproductive or thermoregulatory stress result in desperate conditions under which an animal will be more likely to exploit a SM with potential for toxicity or therapy. Specifically, animals with long-term associations with SMs are more likely to exploit SMs for chronic challenges. For example, insects that exploit SMs in defending against predators typically have long-term associations with the SM (e.g., dietary specialization). These long-term associations are likely linked to resistance via mechanisms that reduce absorption, increase detoxification, or isolate SMs to specific tissues, thus reducing the toxicity of the SM (i.e., wide therapeutic index) in the co-evolved insect. We also predict that long-lived, social animals, which frequently sample their environment, are likely candidates to learn how to exploit SMs for benefit. However, we predict that the ''social model'' of exploitation may be rare, as it relies on several complex factors. First, the SMs must have a relatively wide therapeutic index, otherwise animals would simply avoid the SM. Second, the intake of the SMs with potentially therapeutic properties must coincide with the challenge, thus sampling frequency should increase with increasing challenge. Third, the animal must associate the intake of a particular plant with a reduced cost of a challenge, thus SMs that provide an immediate treatment for the challenge are more likely to be exploited. Forth, the animals must be able to titrate the intake of an SM to remain above the therapeutic concentration, but below the toxic concentration (Fig. 1). Finally, conditions should favor the transfer and maintenance of knowledge of which SMs should be exploited to treat specific challenges, e.g., in social animals that reside in stable environments. We also urge researchers to investigate the absorption, distribution, detoxification and elimination of the SMs by the exploiter (see Sotka et al. this issue and Sorensen et al. 2006) to verify that the SM is distributed to the intestine or blood. Even small samples of the blood, urine and feces can be sufficient to verify the distribution of SMs or their derivatives. Studies of biodistribution of SMs are needed to verify the link between the intake of an SM, the concentration,

and the subsequent toxicity or treatment of the challenge.

Ecologists should consider conducting experimental studies to validate the exploitation of SMs. Studies involving controlled feeding can be used to investigate self-medication of natural SMs under varying costs of the challenge while also varying the therapeutic index of single SMs and combinations of SMs or plants containing an array of SMs for treatment. In addition, studies of feeding should link the extent of absorption, detoxification and potential toxicity of SMs and their derivatives to the cost of various challenges. Finally, researchers should be cognizant of how nutrients influence homeostasis and interact with challenges and SM toxicity (Raubenheimer 1992). Biochemical complementarities and sequence of ingestion both influence the degree to which herbivores can titrate concentrations of SM in relation to their homeostatic benefit (Welch et al. 2009).

Broad application

The homeostatic perspective allows researchers to maintain a holistic understanding of animal behavior in response to costly challenges. By thinking of SMs as a resource that can help animals maintain homeostasis, we are able to identify novel explanations for foraging behavior. The homeostatic perspective will advance our understanding of what drives foraging behavior and the behavior of animals under challenging insults. We now discuss how the homeostatic perspective can also be broadly applied to improve the management of animals and potentially lead to discovery of new drugs.

Management of animals and landscapes

The major goal in agriculture is to maintain the well-being of livestock while increasing food production. One of the major challenges facing health and production of livestock is parasites. Although humans have used other approaches to reduce parasites, including grazing management, biological control, nutritional supplementation, vaccination, and selective breeding (Stear et al. 2007), they have come with mixed results, and none incorporate the homeostatic perspective: that herbivores can meet their nutritional needs and potentially combat parasites themselves if provided with biochemically diverse foods. Given the evidence that animals can benefit from SMs (e.g., reduced parasite loads), we propose that management practices should offer animals a variety of forages that differ in primary and secondary metabolites. The ability to choose foods enables livestock in confinement, on pastures, and on extensive landscapes to better meet needs for nutrients and to regulate intake of SMs (Provenza et al. 2003, 2007). Providing diverse food options may also benefit wild and captive animal populations. On the island of Bali in Indonesia, a free-ranging population of temple monkeys in the Ubud Sacred Monkey Forest is periodically fed papaya leaves by park staff, a dietary change believed to be responsible for the population's parasite-free status (Aida Rompis, DVM, personal communications to MA Huffman). Although the direct link between the intake of papaya leaves and the lack of infection by parasites is speculative, monkeys at other sites in Bali that do not receive these leaves have high parasite loads (Aida Rompis, DVM) and the use of papaya by indigenous peoples as a treatment for parasites (malaria, amebiasis, intestinal helminths), digestive upsets, and as an antiseptic (Iwu 1993), strengthens these claims. These studies suggest that the health of livestock and wildlife could be improved by an appreciation for both the toxic and beneficial properties of SMs in diverse foods.

Overlap with exploitation of SMs by humans

There is substantial overlap between both the explanation for SM exploitation and the fate and mechanism of action of SMs in humans and animals. First, it is in the best interest of both humans and animals to maintain homeostasis. Second, humans experience costs of parasites (Pullan and Brooker 2008), reproduction (Reed et al. 2008) and thermoregulation (Gentilello 1995). Third, humans exploit many of the same plants as treatments for the same challenges as do animals (Huffman et al. 1998). For example, the SMs in juniper thought to provide thermal benefits to woodrats in the winter through reduced heat dissipation are the same SMs in turpentine that are used to induce hyperthermia in humans (Hernandez-Espinosa et al. 2007). In addition, there are many examples of plants whose medicinal value to humans was discovered by observing self-medication in wild animals (Huffman 2007a), further revealing the similarities between humans and animals. Finally, there is conservation of the mechanisms used to absorb and detoxify SMs across species (Saier and Paulsen 2001; Sorensen and Dearing 2006). Furthermore, the genetic conservation of receptor targets across species means that SMs may bind to the same receptor targets in a variety of species, thus enabling many animal models to predict effects of drugs on humans (McGrath and Li 2008). For example, the

cannabinoid receptors are not only highly conserved in vertebrates, but their function (e.g., in reproduction and neurotransmission) and ligands are similar across species (Lam et al. 2006; Chianese et al. 2008). This overlap in use and mechanisms of action of SMs allows the knowledge of the exploitation of SMs in animals to advance discovery of drugs.

Discovery of bioactive SMs

Modern discovery of drugs is based on the identification of generally small molecules that either inhibit, activate or otherwise modulate a certain macromolecule (e.g., protein, enzyme, receptor) crucial for a given pathogenic condition (Koehn and Carter 2005). Organisms from microbes to humans share a great deal of biochemical architecture. In particular, proteins (e.g., primary metabolites) can be found in all organisms in one form or another, although their homologies might differ slightly. Because many SMs are believed to have evolved within the context of their interaction with the proteins of both lower (bacteria–fungi) and higher organisms (herbivores) at the biochemical level in ways that deter predators (e.g., binding to taste receptors) or inhibit the growth of a competing organism (e.g., direct toxicity), they are able to interact with human proteins bearing a homologous domain, active site or regulatory region. This evolutionary history makes SMs 'privileged structures' and underlies their drug-like properties and high biomedical potential. SMs are more similar in their chemical diversity to current drugs on the market than are compounds obtained through combinatorial chemical synthesis (Feher and Schmidt 2003; Koehn and Carter 2005; Larsson et al. 2007). Thus, despite the significant advances in organic chemistry and the availability of large libraries of synthetic chemicals, SMs are still an important source for novel drugs (Newman and Cragg 2007; Butler 2008; Harvey 2008).

Sessile organisms, such as plants or invertebrates (e.g., larvae) that lack physical defense systems, and thus rely on SMs as chemical defenses, are great sources of new drugs. However, the immediate question that arises is how to discover SMs that have medicinal properties. There are several classical approaches for choosing plants or other biological material that may contain new SMs. In the random approach, all available plants are collected for screening, irrespective of any previous knowledge of the folkloric use of the plant. This is commonly used when little information is available on the plant or organism of interest. However, it is estimated that there are more than 400,000 plant species worldwide (Govaerts 2001) and, hence, many researchers and pharmaceutical companies seek ways of reducing the number of plants to be screened. Taxonomical or chemotaxonomical approaches attempt to focus screening within only certain plant genera or plants containing certain classes of SMs. The ethnopharmacological approach provides an additional type of focused screening by giving credence to existing oral or written information on the traditional use of a plant against a human disease. This is a common approach in societies where traditional medicines constitute an important form of healthcare (Ayurveda, TCM, Campo Medicine; also see Kingston et al. 1999; Lewis et al. 1999). However, cultural, governmental and intellectual property issues associated with exploiting ethnobotanical knowledge limits commercial interest in ethnomedicine (Soejarto et al. 2005). Because therapeutic compounds in nature are becoming increasing difficult to discover by these traditional approaches (Clardy and Walsh 2004), there is a pressing need for novel ways to identify and screen diverse sets of chemicals.

We propose that integrating the fields of ecology (both physiological and chemical) and pharmacology can provide an additional and efficient route to discover medicinally active SMs. Much as ethnomedicine relies on knowledge of traditional or indigenous health-care practices, the ''Pharm-Ecology'' approach relies on the identification, description and knowledge of potentially valuable plant–animal interactions. Broad understanding of the chemical interactions between SMs and the physiological and behavioral responses of herbivores to these compounds may expedite discovery of drugs and provide a better understanding of the mechanisms by which bioactive molecules are therapeutic and/or toxic. Although our ancestors responsible for many traditional medicines may have been practicing ecological bioprospecting since ancient times, scientific researchers have only recently embraced this approach. As described earlier, many examples of ecological bioprospecting exist in the study of primate behavior and of the ecology of parasites (Huffman 1997, 2007b; Engel 2002). In addition, the use of ecological theory in bioprospecting has improved the success rate of discovering useful pharmaceuticals in tropical plants (Coley et al. 2003; Helson et al. 2008). With an enhanced awareness of the possible usefulness of SMs for discovering drugs, ecologists could highlight ''case studies'' of plant–herbivore interactions, specifically the exploitation of SMs, to alert pharmacologists to the presence of potentially valuable leads for new drugs.

Conclusion

Agronomists and ecologists alike have come to view SMs as defenses against herbivory because SMs limit intake. Thus, we know little about how herbivores might benefit from SMs, despite their use by humans for medicinal benefit. Using homeostasis to understand the choice of diets and the behavior of animals under various challenges provides a novel perspective that goes beyond the traditional explanations that foraging behavior is guided by selection for nutrients and avoidance of SMs. This perspective may explain dietary changes occurring under different pressures arising from predation and infection, during reproduction, under changing temperatures and, potentially, at times requiring heightened alertness. Examples of exploitation of SMs by animals, although often correlative, suggest that animals may not always choose to avoid SMs, but may actually exploit the biological activity of SM in ways that reduce costly challenges. There is great need for controlled studies that can strengthen existing correlations and anecdotal evidence of the exploitation of SMs and test predictions on how exploitation of SMs by animals evolved. We recognize that some systems will be more amenable than others to experimental manipulations, but behavioral ecologists should attempt to design studies of feeding that provide a choice of potentially therapeutic SMs under varying types and costs of challenges. We also stress that studies should consider the therapeutic index of SM in species of interest and remain cognizant that the therapeutic index for the same SM can vary across species due to differences in the capacity to absorb, detoxify and eliminate the SM and due to differences in receptor binding.

Understanding the probability of exploitation of SM by animals can be used to make and test several predictions:

(1) If animals consume a potentially toxic SM that is normally avoided, then the cost of a particular challenge is greater than the cost of toxicity from the SM.

(2) The greater the cost of the challenge required before consuming a SM with potentially therapeutic properties, the narrower the therapeutic index is for that SM.

(3) The exploitation of SMs for less costly challenges suggests that the SM has a very wide therapeutic index in general or that the animal exploiting the SM has high capacity to detoxify and eliminate the SM such that the therapeutic index in the exploiting animal is wide.

We hope this review encourages researchers to test the various predictions we have proposed about the exploitation SMs by animals and to consider how to apply this new perspective and knowledge to advance the management of animals and the discovery of drugs.

Funding

The opportunity to present and synthesize ideas related to this work was supported by the National Science Foundation (0827239 to J.S.F.), the Society of Integrative and Comparative Biology, and Agilent Technologies, Inc, Santa Clara, CA, USA.

Acknowledgments

We would like to thank all the participants in the Pharm-Ecology Symposium for providing their ecological, physiological, chemical, and pharmacological insight to move research on plant–herbivore interaction into new directions. We thank SICB for logistical support, especially Dr Eduardo Rosa-Molinar, the program officer, and Sue Burk for providing an opportunity to participate in the symposium and for organizing the SICB meeting.

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